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The effect of grapefruit extract and temperature abuse on growth of *Clostridium perfringens* from spore inocula in marinated, *sous-vide* chicken products ☆

Vijay K. Juneja [a,*], Xuetong Fan [a], Aida Peña-Ramos [b], Martha Diaz-Cinco [b], Ramon Pacheco-Aguilar [b]

[a] *Microbial Food Safety Research Unit, US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA*
[b] *Centro de Investigacion en Alimentacion y Desarrollo, A.C., Hermosillo, Sonora, Mexico*

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Abstract

Clostridium perfringens growth from a spore inoculum was investigated in vacuum-packaged, cook-in-bag, marinated chicken breast that included additional 1.0% NaCl. The packages were processed to an internal temperature of 71.1 °C, ice chilled and stored at various temperatures. The total *C. perfringens* population was determined by plating diluted samples on tryptose–sulfite–cycloserine agar followed by anaerobic incubation for 48 h at 37 °C. At 19 °C, *C. perfringens* levels were consistently about 2.5 $\log_{10}$ CFU/g until 9.5 h regardless of the presence or absence of Citricidal®. However, storage of the unsupplemented marinated chicken samples and those with 50 or 100 ppm Citricidal® samples at 25 °C for more than 6 h resulted in rapid growth of *C. perfringens,* exhibiting 2–3 $\log_{10}$ CFU/g increase at 7 h. Citricidal® at 200 ppm significantly ($p<0.05$) reduced the growth of *C. perfringens* at both 19 and 25 °C. The *D*-values obtained at 90 °C were significantly decreased ($p<0.05$) from 14.07 (no Citricidal®) to 9.20 min (200 ppm Citricidal®). Supplementing marinated chicken products with Citricidal® and the temperature abuses had no consistent effect on color, shear force or lipid oxidation. However, the organism may grow to unsafe levels if *sous-vide* products are poorly handled or temperature abused for a relatively long period. An extra degree of safety may be assured in such products by supplementation with 100 or 200 ppm Citricidal®.
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Keywords: *Clostridium perfringens*; Chicken; Spores; Growth; Citricidal®

Industrial relevance: Since temperature abuse is a common occurrence during trasportation, distribution, storage or handling in grocery stores or by consumers, an extra degree of safety may be assured in marinated chicken products by supplementation 100–200 ppm Citricidal. In such products, the temperature abuse should not have any consistent effect on color, shear force or lipid oxidation of products.

1. Introduction

Consumers have been demanding fresh tasting, high-quality, low salt, preservative free, convenience meals which require minimal preparation time. This demand by consumers has resulted in increased production of minimally processed, ready-to-eat, extended shelf-life refrigerated foods, which include *sous-vide* (under vacuum) processed food products. *Sous-vide* food processing is an advanced method of cooking whereby fresh food is vacuum sealed in heat-stable, high barrier plastic pouches or films, and then cooked (pasteurized) to time and temperature combinations sufficient to destroy vegetative pathogens but mild enough to maximize the sensory characteristics of the product (Peck, 1997; Rhodehamel, 1992). After cooking, the specific food is chilled rapidly to avoid germination and outgrowth of surviving bacterial spores, stored refrigerated, and reheated before consumption. *Sous-vide* processed products are mainly used in mass catering and restaurants (Martens, 1996) and have a shelf life of less than one week to six weeks

☆ Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

* Corresponding author. Tel.: +1 215 233 6500; fax: +1 215 233 6697.
E-mail address: vjuneja@errc.ars.usda.gov (V.K. Juneja).

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depending upon the severity of the cooking or pasteurization step and storage temperatures (Schellekens, 1996).

Commercial application of *sous-vide* processed foods in North America has been limited. This is because the safety and preservation of *sous-vide* foods rely mainly on a mild heat-treatment and chilled storage, and there is a requirement for long shelf-lives. Accordingly, the microbiological safety and preservation of the *sous-vide* processed foods are being questioned and warrant a critical evaluation of the process. Concerns have been expressed about the serious public-health risks associated with these foods. The concerns regarding *sous-vide* foods are justified for a variety of reasons: The thermal process is not designed to destroy bacterial spores or to result in commercial sterility, and more significantly though, since the spoilage microflora which play a significant role causing deterioration and spoilage are inactivated, the foods may become toxic while remaining organoleptically acceptable. These products are generally formulated with little or no preservatives and frequently do not possess any intrinsic inhibitory barriers (pH, a_w, or NaCl) that either alone or in combination would inhibit growth. The *sous-vide* foods are vacuum packaged in high barrier or air impermeable flexible bags or pouches/packages (to extend the shelf life from a nutritional and sensory point of view), which provides a favorable growth environment for anaerobic psychrotrophic pathogens, before being subjected to pasteurization treatment. These products require refrigeration at temperatures as low as 3.3 °C or below from production to consumption to prevent spoilage and assure microbiological safety. Sufficient evidence exists to document that temperature abuse is a common occurrence at both the retail and consumer levels. According to National Food Processors Association (NFPA, 1988), manufacturers should assume that temperature abuse will occur at some point during the distribution of a refrigerated food product. Surveys of retail food stores and consumer refrigeration units have revealed that holding temperatures of >10 °C are common (Daniels, 1991; Hutton, Dhehak, & Hanlin, 1991). Thus, it is unrealistic to rely on refrigeration for the safety of *sous-vide* processed foods.

The fastest growing ethnic market in western United States today is Mexican foods. This market has developed beyond tortillas and tacos, and U.S. consumers are now demanding fully cooked Mexican entrees with authentic flavor, texture and appearance, while at the same time, providing ease of preparation, and have a shelf life sufficient for fresh distribution. Mexican meat processors are well aware of this trend, and have targeted the U.S. to export their products. The main obstacle that processors in Mexico have to overcome is preserving the original fresh-cooked taste during distribution and display in stores. Some meat industries have turned to *sous-vide* as a good processing alternative to meet these requirements. Most of Mexican meat products are usually prepared with a combination of two or more herbs and spices. Many herbs and spices have been reported to possess antimicrobial activity against a variety of food pathogens (Shelef, 1983; Yin & Cheng, 2003; Zaika, 1988). It is important to mention, also, that some of these spices and herbs have been reported as a potential source for *Clostridium perfringens*, thereby contaminating the processed foods (Garcia, Iracheta, Galvan, & Heredia, 2001; Rodriguez-Romo, Heredia, Labbe, & Garcia-Alvarado, 1998).

Several studies have suggested grapefruit extracts have antimicrobial activity. Spraying of *Salmonella*-inoculated chicken skin with 0.5% grapefruit seed extract significantly reduced the population of the pathogen (Xiong, Slavik, & Walker, 1998). Treatment of alfalfa sprouts with Citricidal® (100 ppm) for 5 min reduced native coliforms by 1–2 log CFU/g and reduced *Salmonella* and *Vibrio* by up to 1.5 log CFU/g (Castro-Rosas and Escartin, 1999). Fett and Cooke (2003) demonstrated that Citricidal® and several other citrus-related products at a concentration of 20,000 ppm reduced population of *Escherichia coli* O157:H7 and *Salmonella* artificially inoculated on alfalfa seeds. According to the manufacturer (Nutriteam, Ripton, Vt.), Citricidal® is a broad spectrum antimicrobial compound synthesized from the seeds and pulp of grapefruit. The active ingredients are quaternary ammonium compounds.

Since temperature abuse is a common occurrence at both the retail and consumer levels, the Refrigerated Food and Microbiological Criteria Committee of the NFPA (1988) has recommended that additional safety barriers be incorporated in refrigerated foods. To our knowledge, the effects of the *sous-vide* process on *C. perfringens* spores and the fate of spores during storage of Mexican foods treated by the *sous-vide* have not been reported. Therefore, the present work was undertaken (a) to investigate the effect of static temperature abuse of refrigerated vacuum-packaged, cook-in-bag marinated chicken breast meat samples on *C. perfringens* spore germination and multiplication of vegetative cells, (b) to determine if Citricidal®, a grapefruit extract (NutriTeam, Inc. Ripton, Vt.), would suppress growth of *C. perfringens* spores in *sous-vide* cooked chicken meat, (c) to define the heat treatment required to inactivate *C. perfringens* spores, likely to be present in raw foods, and (d) to determine whether Citricidal® and temperature abuse will impact sensory quality.

2. Materials and methods

2.1. Test organisms and spore production

C. perfringens strains NCTC8238, NCTC8239, and ATCC10288 from the Microbial Food Safety culture collection were maintained as sporulated stock cultures in cooked meat medium (Difco Laboratories, Detroit, MI). An active culture was prepared in freshly prepared fluid thioglycollate medium, and sporulation was carried out in Duncan and Strong sporulation medium as previously described (Juneja, Call, & Miller, 1993). After the spore crop of each strain had been washed twice and resuspended in sterile distilled water, the spore suspensions were stored at 4 °C. A spore cocktail containing all three strains of *C. perfringens* was prepared immediately prior to experiments by mixing equivalent numbers of spores from each suspension.

2.2. Source, preparation and inoculation of meat

Thin sliced boneless and skinless fresh chicken breast cutlets (Perdue brand) were obtained from a local retail market and

frozen (−5 °C) until use (approximately 40 d). Forty grams of Dona Maria mole sauce (Mexicorp Foods, El Paso, TX) supplemented with additional 1% salt was weighed in filter stomacher bags (SFB-0410; Spiral Biotech., Bethesda, MD) and then 60 g of chicken meat was added (Meat and sauce — 60:40 ratio). Thereafter, 0, 50, 100, or 200 ppm Citricidal® liquid concentrate (60% grape fruit extract and 40% glycerin) was added to the bags before inoculating with the heat-shocked (75 °C/20 min) *C. perfringens* spore cocktail so that the final concentration of spores was approximately 3 $\log_{10}$ CFU/g. The contents of each bag were mixed with a stomacher for 2 min. The pH of the sauce and poultry meat mixture was determined using a Sensorex semi-micro combination electrode (A.H. Thomas, Philadelphia, PA) attached to an Orion model 601A pH meter. Negative controls consisted of bags containing uninoculated samples. The bags were placed in 7″ × 8″ plastic barrier bags (Koch Model 01 46 09, Kansas City, MO). The oxygen transmission rate of the nylon/polyethylene film was 3.5 cm^3/100 in.2 in 24 h measured at 75 °F and 75% relative humidity. The bags were evacuated to a negative pressure of 1000 mb and heat sealed using a Multivac Model A300/16 gas packaging machine (W. Germany).

2.3. Cooking, storage, temperature abuse, and sampling protocols

The samples were processed to a final cooking temperature of 71.1 °C increased linearly in a period of 1 h and quickly cooled in an ice slurry. The inoculated samples were stored at 4, 19, and 25 °C. Samples stored at 25 °C were analyzed at 0, 3, 4.5, 6, 7, 9, 10.5, 13 and 16 h, those at 19 °C were analyzed at 0, 3, 6, 9.5, 13, 18, 21, 48, 96 and 240 h and the samples stored at 4 °C on day 7, 14, 21, and 28.

2.4. Bacterial enumeration procedure

On the scheduled sampling day, samples were removed and enumerated for total *C. perfringens* population by spiral plating (Spiral Systems Model D plating instruments; Cincinnati, OH) on tryptose–sulfite–cycloserine (TSC) agar as described previously (Juneja, Marmer, & Miller, 1994a). The total *C. perfringens* population was determined after 48 h of incubation at 37 °C in an anaerobic chamber. In addition, a portion of both uninoculated raw meat and sauce mixture was used to verify the absence of naturally occurring *C. perfringens*.

2.5. Thermal inactivation and enumeration

Heat-shocked *C. perfringens* spores inoculated in chicken meat samples, both with and without 200 ppm Citricidal®, were vacuum packaged. Thereafter, the bags were placed in a basket and fully submerged in a temperature controlled water bath (Exacal, Model Ex-251HT, NESLAB Instruments, Inc., Newington, NH) stabilized at 90 °C. The temperature was continuously monitored by two copper–constantan thermocouples inserted, prior to heat sealing, at the center of two uninoculated bags. The thermocouple readings were measured and recorded using a Keithyl–Metrabyte data logger Model DDL 4100 (Tauton, MA) connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings were averaged to determine the bag internal temperature. Come-up times, which were negligible, were included as part of the total heating time when these were used to calculate the *D*-values. Two bags for each replicate were then removed at designated time intervals. After removal, bags were immediately plunged into an ice-water bath, then analyzed within 30 min by the procedure described above. For each replicate experiment performed in duplicate, an average CFU/g of four platings of each sampling point was used to determine the *D*-values.

2.6. Assessment of organoleptic attributes

Only uninoculated samples were used in this part of the study. Samples with or without those supplementation with 200 ppm Citricidal® were assessed for appearance (color), texture and lipid oxidation. The sauce and chicken in stomacher bags were agitated so that the sauce was in good contact with all areas of the chicken breast. The bags were heat sealed and placed in a 4 °C refrigerator for 5–7 d before being cooked. The samples after cooking were incubated at 19 °C for 13 h, and 25 °C for 7 h. Each treatment was replicated twice (each replicate in duplicated samples). Texture, color, and lipid oxidation were measured immediately after the temperature abuse conditions.

2.7. Measurement of lipid oxidation

Lipid oxidation was measured using the thiobarbituric acid (TBA) assay. Ten grams of chicken breast samples were homogenized with 20 ml 0.5 M phosphate (pH 3.0) buffer containing 0.01% 2(3)-*tert*-butyl-4-methoxyphenol (BHA) using a homogenizer (Virtishear, VirTis, Gardiner, N.Y.) at a speed setting of 70 for 2 min on ice. The homogenate was filtered through a Whatman 113V paper filter (Whatman, Clifton, NJ). A 1.6 ml aliquot of filtrate was then added to a test tube containing 1.6 ml of 15% (w/v) trichloroacetic acid and 0.65% TBA. Samples were then mixed vigorously, heated at 95 °C in a water bath for 25 min, cooled and centrifuged at 1300 *g* for 10 min. The supernatant was then filtered through a 0.45 μm Millex®-HX PVDF membrane filter (Millipore, Bedford, MA). Absorbance at 532 nm was monitored using a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). TBA reactive substance (TBARS) values were expressed as malondialdehyde (MDA) equivalent and calculated from a standard MDA curve. There were four measurements (one from each sample).

2.8. Color measurement

The chicken samples were rinsed with tap water before color and texture measurements. Color (*L**, *a** and *b**) analysis was performed using a Hunter Miniscan XE meter (Hunter Laboratory, Inc., Reston, VA). *L** values indicated darkness to lightness; *a** indicated greenness to redness; *b** values indicated blueness to yellowness. The meter had been calibrated using

black and white standard tiles. Illuminate D65, 10° standard observer, and a 2.5 cm port/viewing area were used. There were two measurements made on each sample (one measurement per side of a chicken slice), and a total of 8 measurements per treatment.

2.9. Texture measurement

Samples for shear force analysis were prepared and measured according to Lyon and Lyon (1998). Two 1.9-cm wide strips were obtained from each cooked piece of chicken breast. The thickness of breast strips was measured using a digital caliper. A Warner–Bratzler (WB) blade attached to a TA-XT2I texture analyzer (Stable Micro System, Surry, UK) was used. This blade was a rectangular blade with an inverted V cut in the bottom edge. The height of the TA-WB blade was set 55 mm above the base. The blade traveled at 2 mm/s to the sample that was positioned on the slotted platform with muscle fibers perpendicular to the blade. As the blade traveled down and through the slot, the samples were sheared and maximum force was recorded. The force was calculated and expressed as shear force/unit thickness (g/mm).

2.10. Statistical analysis

Analysis of variance was done using SAS (SAS Institutes Inc., 1989, Cary, NC). Bonferroni mean separation test was used to determine significant differences ($p<0.05$) among means (Miller, 1981).

3. Results and discussion

The pH of the marinated chicken breast used in the study was about 5.0. The cumulative changes in average $\log_{10}$ CFU/g for *C. perfringens* at 19 and 25 °C are shown in Tables 1 and 2, respectively. At 19 °C, *C. perfringens* levels were consistently about 2.5 $\log_{10}$ CFU/g until 9.5 h regardless of the presence or absence of Citricidal®. All sampling times less than 9.5 h exhibited population densities that were not significantly different from one another ($p\geq 0.05$). However, a rapid increase in the pathogen population by approximately 3.0 $\log_{10}$ CFU/g was observed at 13 h in marinated chicken breast control samples and in those samples supplemented 50 or 100 ppm Citricidal®. In these samples, *C. perfringens* spores germinated and grew from 2.5 $\log_{10}$ CFU/g to approximately 6 $\log_{10}$ CFU/g after 13 h (Table 1). Citricidal® at 200 ppm significantly ($p<0.05$) reduced the growth of *C. perfringens*. In samples supplemented with 200-ppm Citricidal®, growth of *C. perfringens* was slow until 21 h. Even at 48 h, *C. perfringens* numbers increased by about 3 $\log_{10}$ CFU/g in samples with 200 ppm Citricidal® compared to about 5 logs increase in those samples that contained less that 200 ppm or no Citricidal®. It is not feasible to compare data published in the literature on the germination and growth of *C. perfringens* in a chicken product because of the unavailability of a common test temperature used. In a study by Juneja, Marmer, and Miller (1994b), *C. perfringens* aerobic growth in cooked turkey was relatively slow at 15 °C and mean $\log_{10}$ CFU/g increased aerobically by 4–4.5 logs by day 8. In another study, growth at 15 °C under atmospheres containing 20% O_2 was less than 3 logs by day 7 (Juneja, Marmer, & Call, 1996). *C. perfringens* spores germinated and grew from 2.25 to approximately 5 $\log_{10}$ CFU/g after 4 d in cooked ground turkey containing 1% salt at 15 °C (Juneja & Marmer, 1996).

During storage at 25 °C, growth of *C. perfringens* was slow in marinated chicken breast supplemented with various levels of Citricidal® until 6 h, since the population densities for all treatments consistently ranged from 3.2 to 3.6 $\log_{10}$ CFU/g (Table 2). Interestingly, storage of the unsupplemented marinated chicken samples and those with 50 or 100 ppm Citricidal® samples for more than 6 h resulted in rapid growth of *C. perfringens*, exhibiting 2–3 $\log_{10}$ CFU/g increase at 7 h. In case of samples that contained 200 ppm Citricidal®, the total cell population at 7 h increased by only 1.0 $\log_{10}$ CFU/g (2.24 to 3.51 $\log_{10}$ CFU/g). However, a parallel increase in the growth of *C. perfringens* with the increase in storage time was observed after 7 h of storage, though the levels were consistently low in samples containing 200 ppm Citricidal® compared to the other treatments that either were not supplemented or contained up to 100 ppm Citricidal®. In a study by Juneja and Marmer (1996),

Table 1
Clostridium perfringens cumulative change in growth ($\log_{10}$ CFU/g) during storage at 19 °C from a spore inoculum in vacuum-packaged, cook-in-bag marinated chicken breast that included 1.0% sodium chloride and 0, 50, 100, or 200 ppm Citricidal®

Time (h)	Citricidal® (ppm)			
	0	50	100	200
3	0.32	0.01	0.55	0.06
6	0.59	0.16	0.11	0.24
9.5	0.52	0.44	0.03	0.25
13	2.59	3.24	2.26	0.35
18	4.22	4.11	4.13	0.62
21	3.92	4.49	3.79	0.52
48	4.93	5.17	4.89	3.09
96	4.87	4.71	4.57	4.30
240	4.00	4.31	4.55	4.26

cfu/g (day X) — cfu/g (day 0) at each sampling time.
Data expressed in $\log_{10}$ cfu/g.

Table 2
Clostridium perfringens cumulative change in growth during storage at 25 °C from a spore inoculum in $\log_{10}$ CFU/g in vacuum-packaged, cook-in-bag marinated chicken breast that included 1.0% sodium chloride and 0, 50, 100, or 200 ppm Citricidal®

Time (h)	Citricidal® (ppm)			
	0	50	100	200
3	0.09	0.17	0.21	0.08
4.5	0.25	0.40	0.87	0.30
6	0.84	0.97	0.99	0.59
7	2.76	3.11	3.00	1.01
9	2.64	2.71	3.60	2.02
10.5	3.33	3.49	3.97	2.77
13	4.24	3.93	4.74	3.44
16	4.46	4.40	4.46	4.07

cfu/g (day X) — cfu/g (day 0) at each sampling time.
Data expressed in $\log_{10}$ cfu/g.

C. perfringens spores germinated and grew at 28 °C from 2.25 to approximately 5 $\log_{10}$ CFU/g after 12 h in cooked ground turkey containing no salt. Addition of 1–2% salt in turkey inhibited the growth and the levels were less 4.0 $\log_{10}$ CFU/g after 12 h of storage at 28 °C.

During storage at 4 °C, *C. perfringens* growth from a spore inoculum was not observed in marinated chicken samples, regardless of Citricidal® concentration (data not shown). Similar observations were reported by Juneja and Marmer (1996), who cooked *C. perfringens* inoculated turkey in a water bath to an internal temperature of 71.1 °C and stored it at 4 °C.

The National Food Processors Association (NFPA, 1988) indicated that manufacturers should assume that temperature abuse will occur at some point during the distribution of a refrigerated food product. The normal pasteurization cooking temperatures applied to *sous-vide* foods may be adequate to destroy the vegetative cells of spoilage and pathogenic food-borne pathogens, but could serve as the spore activation step, if it were not lethal to the spores. Desirable organoleptic attributes of foods are unlikely to be retained if the thermal process is designed to inactivate *C. perfringens* spores. For growth of *C. perfringens* to occur in *sous-vide* foods, four criteria must be met: activation, germination, outgrowth of spores, and vegetative growth. In a study by Barnes, Despaul, and Ingram (1963), about 3% of spores germinated in raw beef without prior heat shock, but almost all germinated after the meat was heated. Spores germinate at a reduced rate without prior heat shock (Craven, 1980).

The thermal resistance of *C. perfringens* spores, expressed in *D*-values, in marinated chicken breast samples with no Citricidal® and those that included 200 ppm Citricidal® at 90 °C was quantified. For inoculated chicken with no Citricidal® heated at 90 °C, *C. perfringens* count decreased by 2.52 $\log_{10}$ (6.72 to 4.20 $\log_{10}$ CFU/g) and by 2.42 $\log_{10}$ after 20 and 28 min of heating, respectively. In contrast, heating inoculated chicken with added 200 ppm Citricidal® at 90 °C for 20 and 28 min resulted in 2.84 and 3.36-$\log_{10}$ reduction in bacterial cells (CFU) per g, respectively. Thus, addition of 200 ppm Citricidal® in chicken rendered the spores more sensitive to the lethal effect of heat. This effect was exhibited by lower level of recovery of heated *C. perfringens* spores and quantified by significantly decreased ($p<0.05$) *D*-values. The *D*-values at 90 °C obtained by linear regression were significantly decreased ($p<0.05$) from 14.07 (no Citricidal®) to 9.20 min (200 ppm Citricidal®).

By selecting a common or a closely related test temperature, it is feasible to compare data published in the literature on the heat resistance of *C. perfringens* spores. In a study by Juneja and Marmer (1996), when the thermal resistance of *C. perfringens* spores (expressed as *D*-values in min) in turkey slurries that included 0.3% sodium pyrophosphate at pH 6.0, and salt levels of 0, 1, 2, or 3% was assessed, the *D*-values at 99 °C decreased from 23.2 min (no salt) to 17.7 min (3% salt). In a beef slurry, the *D*-values significantly decreased ($p<0.05$) from 23.3 min (pH 7.0, 3% salt) to 14.0 min (pH 5.5, 3% salt) at 99 °C. While addition of increasing levels (1–3%) of salt in turkey (Juneja & Marmer, 1996) or a combination of 3% salt and pH 5.5 in beef (Juneja & Majka, 1995) or supplementing chicken with 200 ppm Citricidal® (present study) can result in an increase in sensitivity of *C. perfringens* spores to heat, mild heat treatments given to foods that are packaged and then cooked will not eliminate *C. perfringens* spores. In other words, spores are likely to survive the normal pasteurization/cooking temperatures applied to *sous-vide* foods. In fact, it is practically not feasible to inactivate the spores by heat. Cooking temperatures, if designed to inactivate *C. perfringens* spores, may negatively impact the product quality and desirable organoleptic attributes of foods are unlikely to be retained. Mild heat treatment given to *sous-vide* foods could serve as an activation step for spores. Thereafter, germination and outgrowth of spores, and *C. perfringens* vegetative growth are likely to occur in *sous-vide* foods if the rate and extent of cooling are not sufficient. Differences in *D*-values may be due to strain variation, recovery conditions (including the composition and pH of the medium), the presence of inhibitors, time and temperature of heating and above all, the heating menstruum.

The temperature abuses after cooking had no consistent effect on the darkness of cooked chickens although samples stored at 25 °C for 7 h tended to be darker (lower L^* values). Samples with temperature abuses (25 °C for 7 h or 19 °C for 13 h) generally had lower a^* values (less redness) and b^* values (less yellowness) than those without temperature abuse. But the effect was not always significant (Table 3). Citricidal® had no effect ($p \geq 0.05$) on L^* or a^* values, but decreased b^*

Table 3
Effect of temperature abuse and Citricidal® on color[Z], texture and lipid oxidation of cooked chicken breasts marinated with a Mexican sauce

Treatment	Citricidal® (ppm)	L^*	a^*	b^*	Shear force (g/mm)	TBARS (nmol/g)
None	0	$37.7^{abY} \pm 4.2$	$15.7^{ab} \pm 0.8$	$23.2^{ab} \pm 2.5$	$192.6^{bc} \pm 22$	$21.1^{a} \pm 2.6$
None	200	$39.8^{a} \pm 3.9$	$16.0^{a} \pm 0.9$	$23.7^{a} \pm 2.0$	$202.1^{bc} \pm 46$	$21.5^{a} \pm 2.4$
19 °C, 13 h	0	$37.0^{ab} \pm 2.6$	$14.7^{bc} \pm 0.9$	$22.2^{abc} \pm 1.4$	$181.3^{c} \pm 29$	$22.2^{a} \pm 2.9$
19 °C, 13 h	200	$37.7^{ab} \pm 3.4$	$13.9^{c} \pm 1.4$	$20.0^{d} \pm 1.6$	$207.4^{abc} \pm 71$	$21.6^{a} \pm 2.2$
25 °C, 7 h	0	$35.3^{b} \pm 2.1$	$14.3^{c} \pm 1.2$	$21.5^{bcd} \pm 2.3$	$240.3^{a} \pm 56$	$21.0^{a} \pm 1.1$
25 °C, 7 h	200	$35.9^{b} \pm 2.5$	$14.6^{bc} \pm 1.1$	$20.9^{cd} \pm 1.9$	$220.6^{ab} \pm 43$	$20.1^{a} \pm 1.0$

Chicken breasts marinated with mole sauce containing different levels of Citricidal® were cooked to an internal temperature of 71.1 °C. Quality was analyzed either immediately after cooking (none) or after storage at 19 °C for 13 h or 25 °C for 7 h.

[Z] L^* values indicated darkness to lightness; a^* indicated greenness to redness; b^* values indicated blueness to yellowness.

[Y] Means in the same column with the same superscript are not significantly different ($p \geq 0.05$).

values in samples stored at 19 °C for 13 h. Shear force of chicken was not consistently affected by the temperature abuse or Citricidal®. Citricidal® had no significant effect on shear force in any sample. Neither temperature abuses nor Citricidal® had any significant ($p \geq 0.05$) effect on lipid oxidation.

Our results showed color, shear force and oxidative stability of foods were not consistently affected by the brief temperature abuses. It is known that quality of heat-processed meat dishes is limited mainly by lipid oxidation (Kanner, 1992). Temperature abuse generally increases lipid oxidation that leads to change in flavor and texture, loss of vitamin and essential fatty acids, and oxidation of pigments in cooked meats (Igene & Pearson, 1979). Use of antioxidants or marinating sauce can reduce lipid oxidation in heat processed meat (Guntensperger, Hammerli-Merer, & Escher, 1998; Jadhav, Nimbalkar, Kulkarni, & Madhavi, 1995). The chicken breast used in the present study had been marinated with a Mexican sauce before cooked. The number one ingredient in the sauce is sesame seeds which contain many strong antioxidants such as sesamol, tocopherols, and phenolics (Chang, Yen, Huang, & Duh, 2002; Fukuda, Nagata, Osawa, & Namiki, 1986). The antioxidants in sesame seeds and in other ingredients (such as spices) might eliminate lipid oxidation due to temperature abuse, and consequently reduce color and texture changes.

The organism may grow to unsafe levels if *sous-vide* products are poorly handled or temperature abused for a relatively long period. For vacuum-packaged, cook-in-bag marinated chicken products that included additional 1% NaCl, static temperature abuse to 19 °C for <9.5 h and the abuse to 25 °C for <6 h did not lead to significant *C. perfringens* growth from spore inocula. An extra degree of safety may be assured in such products by supplementation with 100–200 ppm Citricidal®. Additionally, the findings indicate that supplementing marinated chicken products with Citricidal® and the temperature abuses had no consistent effect on color, shear force or lipid oxidation.

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